

Sex Determination in *Xiphophorus (Platypoecilus) maculatus*. III. Differentiation of Gonads in Platyfish from Broods Having a Sex Ratio of Three Females to One Male

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 (Plates I & II; Text-figure I)

THE genetic mechanism for sex determination in the common platyfish, *Xiphophorus (Platypoecilus) maculatus*, is not uniform for all its members. For example, the members of natural platyfish populations from the rivers of Veracruz, Mexico, have a mechanism in which the female is homogametic with reference to the sex chromosomes (XX), and the male is heterogametic (XY). On the other hand, the platyfish from the Belize River, British Honduras, have a sex-chromosomal system in which the male is homogametic (YY) and the female is heterogametic (WY). Since platyfish from Mexico and those from British Honduras are of the same species, it has been possible to mate successfully members of the two geographically isolated and genetically different natural populations in order to evaluate the results obtained from combining the various sex chromosomes. This has been facilitated by use of marker genes for color patterns which are linked to specific sex chromosomes.

Among the gametic recombinations that might be expected from the mating of an XX-XY type of platyfish with one having WY-YY, the union of chromosomes W and X is of special theoretical interest. This is particularly so because when a WX female is obtained, it may be tested by mating it to an XY male. Theoretically, the mating of a WX female and an XY male should produce three types of daugh-

ters (WX, WY and XX) to every one male (XY). It is the purpose of this paper to present the experimental verification of the theoretical results expected, first, by documenting the genetic ratios obtained, and second, by showing that the histological evidence confirms the identity of the sexes as first revealed by the genetic methods of analysis.

The color patterns that serve as indicators of the genes on the various sex chromosomes are composed of large black pigment cells or macromelanophores. These pigment cells in genetically imbalanced fishes are potentially capable of neoplastic growth (Gordon, 1951c). The data to be presented provide further evidence of the strong and definite genetic control of the pattern of development of these important pigment cells and the precise method of their inheritance.

MATERIAL AND METHODS

A word of explanation of the symbols used here for the sex chromosomes may be desirable. The formula XX representing femaleness and XY for maleness is familiar, for it has been used to denote the appropriate sexes in *Drosophila* or in man. The formula WY denoting femaleness and YY denoting maleness is less familiar. They represent a modification of the terms used in referring to the sex chromosomes of birds or moths in which WZ represents femaleness, and ZZ maleness. The reason for substituting the Y for the Z in the platyfish is that there is ample experimental proof that the Z chromosome is synonymous with the Y. This has been demonstrated repeatedly (Gordon, 1946b, 1947a, 1951a) in the platyfish (*X. maculatus*) by mating a homozygous female (XX) of the

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XX-XY stock with a homozygous male (YY) of the YY-WY stock. When the parents are XX and YY, all the F_1 individuals are XY and male.

Historically our earliest knowledge of the sex-determining mechanism in the platyfish was derived from analyses made with members of domesticated strains which were originally taken from "Central America" and shipped to Germany, in 1907. A few years later, about 1911-1912, the aquarium-bred platyfish were exported by German aquarists to the United States. The genetic mechanism for sex determination in the early domesticated platyfish was found by Bellamy (1922), and independently by Gordon (1926, 1927), to be YY (or ZZ) for maleness and WY (or WZ) for femaleness. This corresponds exactly to the system found in members of wild populations of the platyfish recently obtained (1949) from the Belize River in British Honduras. And this suggests that our domesticated stocks originated most probably in the Belize River. It was the custom in the early days of the tropical fish hobby for sailors to bring in specimens for aquarists. Belize, in British Honduras, was a port of some importance in the early 1900's for shipping logwood and mahogany. Sailors doubling as fish collectors probably shipped platyfish from this locality to Germany.

Females of the domesticated stocks of the platyfish (WY) and males of the wild stocks (XY) of the same species from the Rio Jamapa, Veracruz, Mexico, were used in the present analysis. The reaction of each of the various sex chromosomes was followed by "tagging" the Y, X and W by one of a series of dominant genes for macromelanophore patterns. The *Sp* (*spot-sided*) gene was manifested by the presence of large black pigment cells scattered over the sides of the platyfish, the *Sb* (*spotted-belly*) gene by similar cells along the ventral surface of the body and the *Sr* (*striped*) gene by several rows of macromelanophores along the sides. The *Sr* gene in the presence of *Sp* or *Sb* was difficult to detect but this did not interfere with the critical analysis. *Sp*, *Sb*, *Sr* and their multiple recessive gene, +, are alleles. In this series of sex-linked alleles there are two additional genes, *N* and *Sd*, which were not used in the present study (Gordon, 1948).

EXPERIMENTAL RESULTS FROM GENETIC ANALYSES

The domesticated *spot-sided* (*Sp*) platyfish, obtained in 1942 from a tropical fish supply house, were quite similar to those studied by Fraser & Gordon (1929). These authors first showed that the W chromosome may carry dominant genes and this may result in a matro-

clinous type of inheritance. Additional proof for this was obtained in a series of experiments, one of which is as follows:

Domesticated Platyfish (8B Stock)		
Female Parent	P ₁	Male Parent
<i>Spot-sided</i>		<i>Non-spotted</i>
(W) <i>Sp</i> /(Y) +		(Y) +/(Y) +
F_1 : 20 Spotted Daughters, (W) <i>Sp</i> /(Y) +		
25 Non-spotted Sons, (Y) +/(Y) +		
Inbred F_1 produced F_2 :		
10 Spotted Daughters, (W) <i>Sp</i> /(Y) +		
15 Non-spotted Sons, (Y) +/(Y) +		
Inbred F_2 produced F_3 :		
18 Spotted Daughters, (W) <i>Sp</i> /(Y) +		
14 Non-spotted Sons, (Y) +/(Y) +		

The next step was to synthesize a strain in which the females had two dominant alleles, one on the W and a different one on the Y. This was accomplished by use of two domesticated stocks as follows:

Female Parent	P ₁	Male Parent
(8B Stock)		(8Sb Stock)
<i>Spot-sided</i>		<i>Spotted-belly</i>
(W) <i>Sp</i> /(Y) <i>Sp</i>		(Y) <i>Sb</i> /(Y) <i>Sb</i>
F_1 (Pedigree No. 104)		
Daughters		Sons
24 (W) <i>Sp</i> /(Y) <i>Sb</i>		19 (Y) <i>Sp</i> /(Y) <i>Sb</i>

The (W) *Sp*/(Y) *Sb* heterogametic female of the domesticated stock (No. 104) was then mated to an (X) *Sr*/(Y) *Sr* heterogametic male of the wild Rio Jamapa stock. *Sr* is more weakly expressed than the other two genes and this makes the detection of *Sp* and *Sb* relatively easy:

Female Parent	P ₁	Male Parent
Domesticated (Stock No. 104)		Wild (Stock 30 ³)
<i>Spot-sided, Spotted-belly</i>		<i>Stripe-sided</i>
(W) <i>Sp</i> /(Y) <i>Sb</i>	×	(X) <i>Sr</i> /(Y) <i>Sr</i>
F_1 (Pedigree No. 107)		
75 <i>Spot-sided</i>		65 <i>Spotted-belly</i>
Daughters:		Sons:
1. (W) <i>Sp</i> /(X) <i>Sr</i> , or		3. (X) <i>Sr</i> /(Y) <i>Sb</i> , or
2. (W) <i>Sp</i> /(Y) <i>Sr</i>		4. (Y) <i>Sr</i> /(Y) <i>Sb</i>

Since all the *spot-sided* (*Sp*) F_1 individuals were females it may be assumed that some were WX (marked "1") with regard to their chromosomal combinations, and approximately the same number were WY ("2"). Since all the *spotted-belly* (*Sb*) F_1 fish were male it may be assumed that both XY ("3") and YY ("4") sex chromosome combinations represent maleness.

Phenotypically it is impossible to separate either the two genotypes representing the fe-

males (1 and 2) or the two genotypes representing the males (3 and 4). One of the *spot-sided* (*Sp*) females from Pedigree No. 107 was taken at random and mated to a *striped* (*Sr*) male of a known stock from the Rio Jamapa. The experiment may be outlined by indicating the possible mating types as follows:

Female (Stock 107) P_1 Male (Stock 30⁵)
 1. (W)*Sp*/(X)*Sr*, or Rio Jamapa
 2. (W)*Sp*/(Y)*Sr* \times (X)*Sr*/(Y)*Sr*
 F_1 (Pedigree No. 194)

13 Daughters:

9 *Spot-sided* (W)*Sp*/(X)*Sr*, or (W)*Sp*/(Y)*Sr*
 4 *Stripe-sided* (X)*Sr*/(X)*Sr*

5 Sons:

5 *Stripe-sided* (X)*Sr*/(Y)*Sr*

The 3:1 sex ratio (13 females to 5 males) may be explained on the assumption that the female parent of the constitution (W)*Sp*/(X)*Sr* (listed as "1") was the fish mated to the (X)*Sr*/(Y)*Sr* male. This would produce the following three combinations of sex chromosomes: WX, WY and XX, all of which would determine femaleness. The remaining combination, XY, would determine maleness. (Text-fig. 1).

The type of experiment just reported required further substantiation; it was therefore repeated several times. Two *spot-sided* (*Sp*) females of Pedigree No. 194 were selected and bred to their *striped* (*Sr*) male siblings. In presenting the results the parental genotypes, listed below as "A" and "B," will be indicated for convenience in advance of the results obtained from evaluation of the F_1 but, of course, the genotypes of each P_1 female (194-1 and 194-2) were determined by the F_1 data:

"A" *Spot-sided* Female P_1 *Stripe-sided* Male
 (194-1) (194-11)
 (W)*Sp*/(Y)*Sr* \times (X)*Sr*/(Y)*Sr*
 F_1 (Pedigree No. 195):

14 *Spot-sided* 16 *Stripe-sided*
 Daughters: Sons:
 (W)*Sp*/(X)*Sr*, or (X)*Sr*/(Y)*Sr*, or
 (W)*Sp*/(Y)*Sr* (Y)*Sr*/(Y)*Sr*

The sex ratio was 1:1.

"B" *Spot-sided* Female P_1 *Stripe-sided* Male
 (194-2) (194-12)
 (W)*Sp*/(X)*Sr* \times (X)*Sr*/(Y)*Sr*
 F_1 (Pedigree No. 195a):

18 Daughters:

12 *Spot-sided* (W)*Sp*/(X)*Sr*, or
 (W)*Sp*/(Y)*Sr*
 6 *Stripe-sided* (X)*Sr*/(X)*Sr*

8 Sons:

8 *Stripe-sided* (X)*Sr*/(Y)*Sr*

The sex ratio was apparently 3:1.

One final experiment was conducted in order to obtain sufficient numbers of fish in broods showing a sex ratio of three females to one male for a follow-up study of the gonadal development in the very young fishes of various phenotypes. The purpose of making this study was to see if the sex of an individual, as recognized by histological methods, corresponds with its phenotype. In other words, do the various chromosomal combinations determine sexuality of the fishes very early in their development?

A *spot-sided* female (No. 195a-1), presumably (W)*Sp*/(X)*Sr*, of the last brood (Pedigree No. 195a), was mated to its *stripe-sided* male sibling (No. 195a-11), which presumably was (X)*Sr*/(Y)*Sr*. The results obtained were essentially the same as before, as follows:

F_1 (Pedigree No. 195^{2a})

93 Daughters:

59 *Spot-sided*, (W)*Sp*/(X)*Sr*, or
 (W)*Sp*/(Y)*Sr*
 34 *Stripe-sided*, (X)*Sr*/(X)*Sr*

33 Sons:

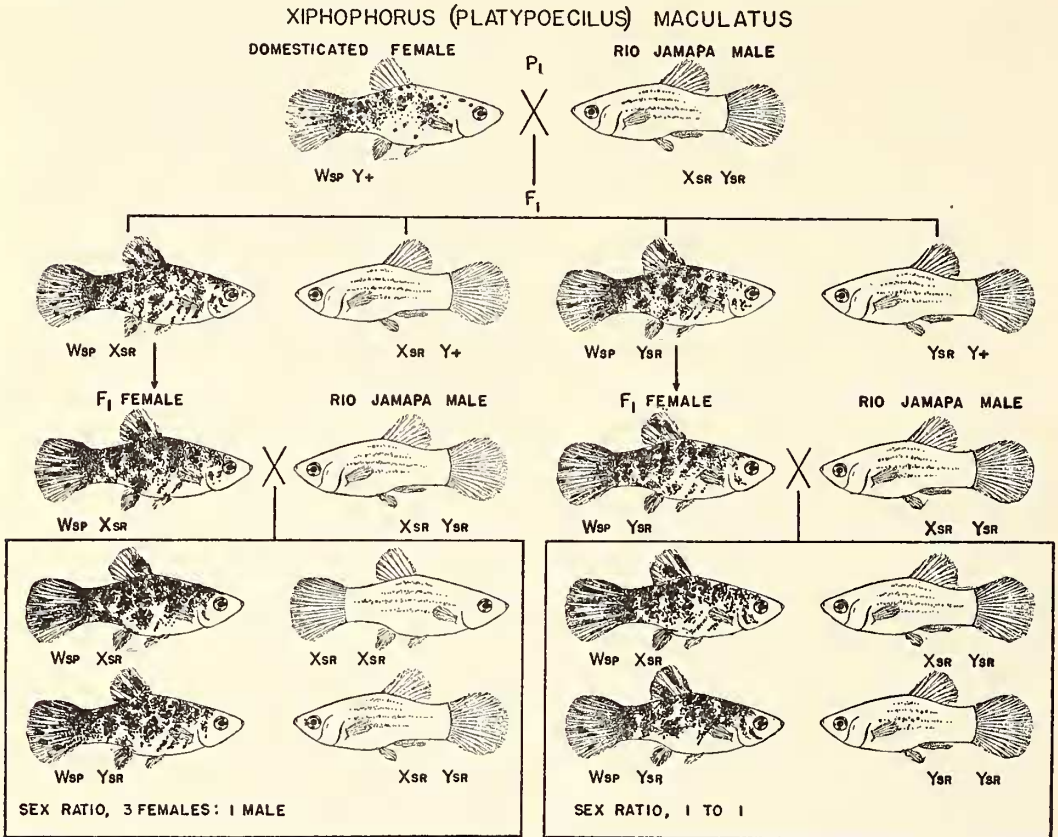
33 *Stripe-sided*, (X)*Sr*/(Y)*Sr*

From the above results the genotypes of the F_1 female and male selected for mating must have been (W)*Sp*/(X)*Sr* and (X)*Sr*/(Y)*Sr*, respectively. Among their 126 F_1 young were 93 females and 33 males, a good three to one ratio.

The above data on sex ratios were obtained, as were all others given previously, by rearing the F_1 fish to four to seven months of age, at which time their sex may be determined by inspection. Externally the sex of mature fish is easily recognized by the differences in the configuration of the anal fin of the male and female. In the male, the single median anal fin is transformed into a thin, grooved, rod-shaped organ or gonopodium which serves, at time of copulation, to transfer spermatophores to the genital aperture of the female. In the female, the anal fin is fan-like, hardly different from that of the young.

DETERMINATION OF SEX IN IMMATURE FISH BY HISTOLOGICAL METHODS

In addition to the 126 fish (Pedigree No. 195^{2a}) reared to sexual maturity, 84 additional young were obtained in three later broods from the same parents (No. 195a-1 and No. 195a-11). Among the young, 40 were *spot-sided* and 44 were *unspotted*. They ranged in size from 9 to 20 mm; their sex could not be recognized with certainty because their anal fins were in an undifferentiated state. On the basis of their color patterns one could predict that the 40 *spot-sided* ones would turn out to be females, as would



TEXT-FIG. 1. Variable Sex Ratios in the Platyfish *Xiphophorus (Platypoecilus) maculatus*. When a spotted female of the domesticated stock WY is mated to a non-spotted (stripe-sided) platyfish from the Rio Jamapa wild stock XY, the sex ratio in the members of the F₁ is 1:1 (lines 1 and 2). Some of the F₁ females contain the sex chromosomes WX while others have WY (line 2). When a WX F₁ female is mated to an XY male (line 3, left), their backcross offspring have a sex ratio of 3 females to 1 male (lines 4 and 5, left). When a WY F₁ female is mated to an XY male (line 3, right), their backcross offspring have a sex ratio of 1:1 (lines 4 and 5, right).

about half of the 44 unspotted ones. The remaining half of the unspotted ones should be male.

In order to determine the sex of the 84 immature fish by histological methods, they were fixed in Bouin's fluid. They were decalcified, sectioned at 10 μ , and stained with Delafield's hematoxylin and eosin.

THE FEMALES

Microscopic examination of the sectioned gonads of the 84 immature fish revealed that 64 of them, 40 spot-sided (*Sp*) and 24 unspotted (presumably *Sr*), had ovaries and the remaining unspotted 20 fish (presumably *Sr*) had testes (Table 1). Thus the histological results confirmed the theoretical expectancy of the three females to one male sex ratio, and in their appropriate phenotypic groupings: all the spotted ones were female; half the unspotted ones were female and half were male.

A special analysis was made to determine the various stages of development of the ovaries in the spot-sided and unspotted females in order to evaluate the relative effectiveness of WX or WY as opposed to that of XX in establishing and regulating the sexual development of the females. A convenient method of rating the developing ovaries was worked out which was based upon (1) the size of the largest ovocyte in the ovary, and (2) the nature of the cytoplasmic substances in the ovocytes with reference to oil and yolk content. This method of analysis was derived from study of a large series of platyfish ovaries, summarized by Berg & Gordon (1952) as follows:

The ovary in the platyfish arises as a paired organ but fuses shortly after its birth or about the time it reaches a length of 7 to 12 mm. In young fish the epithelial cells of the ovarian duct are high columnar. Between these cells occa-

TABLE 1. THE EFFECT OF VARIOUS SEX CHROMOSOME COMBINATIONS ON THE GONADAL DEVELOPMENT IN SIBLINGS OF THE PLATYFISH, *XIPHOPHORUS MACULATUS* OF PEDIGREE NUMBER 195²A

Number of Animals	Standard Length-range	Phenotype	Type of Gonad	Probable Genotype
40	12 to 19 mm	Spot-sided ¹	Ovary	(W) <i>Sp</i> /(X) <i>Sr</i> or (W) <i>Sp</i> /(Y) <i>Sr</i>
24	12 to 20 mm	Not spot-sided ³	Ovary	(X) <i>Sr</i> /(X) <i>Sr</i> ²
20	9 to 15 mm	Not spot-sided ³	Testis	(X) <i>Sr</i> /(Y) <i>Sr</i> ²

¹ *Sp* gene for macromelanophores, producing spots on the sides.
² *Sr* gene for macromelanophores, producing stripes along the sides.
³ In young fish the striped color pattern is hardly developed. This group was recognized by the absence of the spot-sided pattern.

sional germ cells are found. In mature fish the ovary fills the major part of the body cavity. The single ovarian duct originates at the anterior-dorsal part of the ovary. It remains dorsal and straight as it passes caudad; it then curves sharply ventrad in the posterior part of the ovary. Beyond the ovary, the ovarian duct continues posteriorly straight to the urogenital sinus. In mature fish the part of the ovarian duct that lies within the ovary is lined with a single layer of low, cuboidal epithelium.

Ovocytes in various stages of development and degeneration lie in the part of the ovary between the ovarian duct and peritoneal covering. Each ovocyte lies within a follicle which is surrounded by a single-layered theca. A thin layer of well-vascularized stroma lies between the follicles. Oil droplets are seen in ovocytes that are approximately 250 μ in diameter, or greater. Yolk first appears in ovocytes that are 600 μ in diameter. A mature, unfertilized ovum measures 1,500 μ in diameter.

Fishes having ovocytes measuring no more than 200 μ and containing neither oil nor yolk were classified as being in their earliest stage of ovarian development, or group I (Table 2). Those having ovocytes measuring no more than 600 μ , and containing oil droplets only were

classified as being in Stage II. Those having ovocytes measuring up to 1,200 μ and containing oil and yolk droplets were regarded as almost mature and placed in Stage III.

In group I there were four females; in II there were 18; and in III there were 14. The fishes in each group were separated according to their phenotype, spotted or unspotted. Statistical analyses of the relationship between the phenotype of the fish and the condition of their ovaries revealed no significant difference between the spot-sided females and the unspotted ones. It can be inferred, therefore, that in the platyfish the WX or WY chromosome combination has the same effect on the rate of development of the ovary as the XX chromosome complex.

THE MALES

Twenty unspotted immature platyfish of Pedigree No. 195²a, measuring 9 to 15 mm, studied histologically, had testes; they were classified according to their stage of spermatogenesis (Table 3). The state of development of the testes varied from the earliest to the latest. In none was there any evidence of atypical development of the gonad. The variation in the state of development may be attributed to the circumstance that the group represented three differ-

TABLE 2. STAGE OF OVOCYTE DEVELOPMENT IN 36 FEMALES SIBLINGS OF *XIPHOPHORUS MACULATUS* WITH VARYING CHROMOSOMAL SEX DETERMINING MECHANISMS

State of Ovocytes Re: Cytoplasm	Size of Ovocytes	Number of Females	
		Spot-sided (W) <i>Sp</i> /(X) <i>Sr</i> , or (W) <i>Sp</i> /(Y) <i>Sr</i>	Not Spot-sided ¹ (X) <i>Sr</i> /(X) <i>Sr</i>
I No oil, no yolk	20 to 200 μ	3	1
II Oil droplets	200 to 600 μ	7	11
III Yolk droplets	600 to 1200 μ	9	5

¹ The *Sr* fish, as indicated in Table 1, may not show their appropriate stripe-sided color pattern, hence they are referred to as "Not spot-sided."

TABLE 3. STAGES OF SPERMATOGENESIS OF 20 MALE SIBLINGS OF
XIPHOPHORUS MACULATUS OF PEDIGREE NUMBER 1952A

Stages ¹	Characteristics	Number of Males
1: Primordial Germ Cells	Gonad primordia largely composed of stroma Primordial germ cells discrete or in small groups at periphery of testes	5
2: Spermatogonial Acini Few	In each primordium some stroma cells form one large duct Germ cells (spermatogonia) at the periphery of gonad primordia; not more than 8 cells in small acini	2
3: Spermatogonial Acini Numerous	Many branching ducts. Many spermatogonia in each of many acini	7
4: Primary Spermatocytes	Acini contain primary spermatocytes. Comparatively few acini contain spermatogonia	2
5: Secondary Spermatophores	Cords of primary and secondary spermatocytes	0
6: Spermatophores	Spermatophores present in sperm ducts and tubules	4

¹ Stages as defined by Chavin & Gordon (1951).

ently aged broods of fish. This, of course, was also true of the females from the same broods. Since the various stages in spermatogenesis of platyfish were presented by Chavin & Gordon (1951) in the first of this series of papers on sex determination, in this journal, they will not be reviewed here.

DISCUSSION

It is a remarkable fact that two opposing genetic mechanisms for sex determination are found in a single species of platyfish, *Platypoecilus maculatus* (now known as *Xiphophorus maculatus*) according to Gordon & Rosen, 1951, and Gordon 1951b. The acceptance of this fact hinges upon this point: that some members of a species follow the WY female, YY male mechanism, while other members of the same species follow the XX female, XY male system. Bellamy & Queal (1951) have raised the question as to whether these two groups really belong to the same species. They do. In papers by the author (1944a, b; 1946a, b; 1947a, b; 1950) the identity of both the wild and domesticated platyfish in question has been established and clearly stated as *Platypoecilus maculatus*. In this connection, it may be of interest to record the type of genetic mechanism for sex determination in the various wild and domesticated populations that have been analyzed from time to time:

Female Heterogamety (WY), Male Homogamety (YY) in *Xiphophorus maculatus*

1. Domesticated Stocks (Bellamy, 1922 to 1951; Kosswig, 1927 to 1939; Breider, 1936 to 1949; Gordon, 1926 to 1951).

2. Natural Populations from the Belize River (Gordon, 1950).

Female Homogamety (XX), Male Heterogamety (XY) in *Xiphophorus maculatus*

1. Natural Populations from Rio Jamapa (Gordon, 1944a, b; 1946a, b; 1947a).

2. Natural Populations from Rio Papaloapan (Gordon, 1944a, b; 1946a, b; 1947a).

3. Natural Populations from Rio Coatzacoalcas (Gordon, 1951a).

Many reviewers, confronted with the unique fact that both male heterogamety and female heterogamety occur in closely related species of poeciliid fishes (or as in *X. maculatus*, in the same species), have, in a speculative mood, declared that the latter probably evolved from the former. This idea attained some degree of acceptance owing to Winge's (1934) discovery that in *Lebistes reticulatus*, which has the XX female, XY male system, it is possible by artificial selection to get XX males and XY females. Gordon (1951a) pointed out the fallacy of the conclusion based upon this sort of evidence, because when exceptional XX male guppies are mated with equally rare XY females, XX females and XY males are again produced. These results might be expected because in the offspring of sex-reversed parents, the balance between the genes for sex determination in sex chromosomes and the sex genes in autosomes is restored. A somewhat similar example of the genic imbalance in a sexually abnormal male platyfish was described in a prior paper in this series (Gordon & Aronowitz, 1951). A partially

functional male from Rio Jamapa stock that had the two X chromosomes usually characteristic of a female was found by Gordon (1947a). When the exceptional XX male was mated to a normal XX female of a similar stock, all their offspring were normal females, XX, as one would expect on theory. These sex-reversed individuals are important in establishing the type of sex-determination in the members of a particular population but they have little bearing upon the evolution of the system itself.

Bellamy & Queal (1951) have joined others in concluding that in the *maculatus* platyfish, female heterogamety "evolved from male heterogametic forms . . . if . . . male heterogametic *P. maculatus* now exist in certain Mexican river systems."

The facts, of course, are that both sex-determining systems are represented in isolated natural populations of the *maculatus* platyfish. It is more likely that each specialized system (WY-YY and XX-XY) arose independently from an undifferentiated condition, perhaps like the one which exists in the related species, *X. hellerii*. The chief problem is to determine how the force of natural selection has established the XX female, XY male system in the natural populations of the *maculatus* platyfish in three rivers of Mexico, while at the same time it has molded the WY female, YY male system in the Belize River population of the same platyfish species. Perhaps an analysis of the distribution of the frequencies of five sex-linked and seven autosomal genes in the various natural populations of *X. maculatus* may throw some light upon this problem (Gordon & Gordon, unpublished).

From the experimental results indicated in this paper the ratio of three females to one male was obtained by the mating of a "domesticated"—"wild" hybrid female, WX, to a Rio Jamapa, XY, male. A similar three to one sex ratio was obtained by Breider (1942) and recently by Bellamy & Queal (1951) by mating exceptionally rare genetically sex-reversed "domesticated" male platyfish (WY) to normal "domesticated" females (WY). These authors obtained from such matings: three female offspring (1 WW, 2 WY) to one male (YY). Breider did not test the WW females, but Bellamy & Queal did. When WW females were mated to normal YY males, they produced all female platyfish, WY. When WW females were mated to genetically sex-reversed males WY, they, too, produced all female broods, WY and WW.

To understand how an individual with the genetic constitution of a female (XX in the case of the Rio Jamapa stock) is able to function

as a male, the gonad of a genetically sex-reversed XX male was studied by Gordon & Aronowitz (1951). The gonad was abnormal at the time it was examined, but it had been functional for a short period. Bellamy & Queal (1951) found that their exceptional WW females were mostly sterile, and that some had significantly fewer offspring. These details indicate an abnormally functioning gonad. They did not report any follow-up histological studies of the gonads in any of their fishes.

It is interesting that both in the Rio Jamapa stock (XX female, XY male) and in the "domesticated" stock (WY female, YY male) the genetic sex reversals discovered were males only. These exceptional males had the chromosomal constitution of the appropriate type of female, that is, XX male in the former stock and WY male in the latter. Stated another way, no XY females were discovered in the Rio Jamapa stock or YY females in the "domesticated" stocks. (Bellamy & Queal's exceptional females, which he labels "YY" are equivalent to "WW" according to the terms used throughout this paper; WW females are, of course, not sex reversals, but the offspring of a genetically sex-reversed male WY and a normal WY female).

Anyone assigned the task of reviewing the work on genetic mechanisms for sex determination in the *maculatus* platyfish faces a formidable and contradictory array of terms which refer to the various sex chromosomes. For this reason a list of synonyms used by Bellamy, Kosswig, Breider, Castle and Gordon is given in Table 4.

It will be noticed from Table 4, that according to Gordon's terminology, the "domesticated" and the Belize River stock (items 5 and 6) have identical sex-determining mechanisms. This suggests that the "domesticated" stocks may be descendants of fishes originally taken from the Belize River in British Honduras. The history of the "domesticated" platyfish as traced by Gordon (1927) contains nothing which could be used against such a supposition. Gordon indicated that the platyfish most generally studied in the past by geneticists in Europe and in this country were unquestionably those derived from an importation made in 1907 to Germany for aquarium purposes from some unspecified locality in Central America. In 1912, or soon thereafter, the German aquarium-bred platyfish were imported into the United States. The American derivatives of the German stocks of aquarium-bred platyfish were the only ones available to geneticists up to 1932. Bellamy, Kosswig, Breider and Gordon, who analyzed the sex-determining mechanism in the aquarists'

TABLE 4. SEX-CHROMOSOME TERMINOLOGY IN THE PLATYFISH, *XIPHOPHORUS MACULATUS*

Female	Male	Author	Beginning	Stock
1. XY	XX	Bellamy, Breider	1922—	Domesticated
2. WZ	ZZ	Gordon, Kosswig, Breider, Bellamy	1926—	Domesticated
3. XY	YY	Castle	1936	Domesticated
4. XY	YY	Gordon	1946	Domesticated
5. WY	YY	Gordon	1947—	Domesticated
6. WY	YY	Gordon	1950—	Belize River
7. XX	XY	Gordon	1947—	Rio Jamapa, Rio Papaloapan, Rio Coatzacoalcos

platyfish, all found that the male was homogametic and the female heterogametic.

In two expeditions in 1930 and 1932, Gordon (1940) was successful in bringing living platyfish of all known species (*couchianus*, *xiphidium*, *variatus* and *maculatus*) directly from Mexico to the United States. To distinguish the two stocks of *maculatus*, the early laboratory-bred platyfish were called "domesticated," while those brought directly from Mexico to the United States were designated as "wild" stock. Now, however, since there are seven "wild" platyfish populations, each from a different river system, each stock is called by the river of its origin. A study of gene frequencies distributions (Gordon & Gordon, unpublished) of the sex-linked and autosomal patterns has revealed the fact that the Belize River population contains all the color patterns represented by the 1907-1909 importation of platyfish. The platyfish population from the Rio Jamapa at Veracruz and the one from Rio Coatzacoalcos at Puerto Mexico contain genes that were not found in the early German imported platyfish, making it even more unlikely that the fish came from the latter two areas.

If the assumption that the "domesticated" platyfish originally came from the Belize River population is valid, and all the available evidence points to this probability, it is no longer necessary to pay any further attention to Gordon's (1947a) former speculation that in the platyfish the heterogametic female system of sex determination was derived from the heterogametic male system by a process that involved hybridization with the swordtail. In this connection, Bellamy & Queal (1951) also point out that obtaining heterogametic males (WY) and homogametic females (WW) in domesticated stocks does not provide the elements necessary for the reconstruction of the opposite sex-determining system. This is so because when a WY sex-reversed male is mated to a WW female, all the offspring, both WY and WW, are female. Again, the genetic balance between the

sex-linked and autosomal genes for femaleness is restored.

The genic balance theory of sex determination originally proposed by Bridges and later elaborated for fishes by Winge has merit in explaining the sex reversals reported by Bellamy & Queal, as well as those previously found by Breider and by Gordon.

Under normally balanced conditions of sex genes in autosomes and in sex chromosomes, femaleness is determined (1) by XX in wild platyfishes from three Mexican rivers, (2) by WY in those from Belize River, and (3) by WX in some intervarietal hybrids. Maleness is determined (1) by XY in wild Mexican platyfishes and (2) by YY in those from British Honduras.

The three female to one male sex ratio is obtained in intervarietal platyfish hybrids not as a result of sex reversals but rather of the orderly recombinations of appropriate sex chromosomes. The differentiation of the sexes as revealed by histological sections indicates normal processes in action. Platyfishes with WY, WX and XX chromosomes are females, and those with XY are males as early in development as may be determined by histological techniques.

SUMMARY

Members of platyfish (*Xiphophorus maculatus*) populations from the Mexican rivers Jamapa, Papaloapan and Coatzacoalcos, have a genetic mechanism for sex determination in which XX represents femaleness, XY maleness. Members of the same species from the Belize River in British Honduras and those from "domesticated" stocks have a mechanism in which WY represents femaleness and YY maleness.

When a domesticated or British Honduras female platyfish, WY, is mated to a Mexican male, XY, two types of hybrid females, WY and WX, are produced as well as two types of hybrid males, XY and YY. The sex ratio is one to one.

When an intervarietal hybrid female of the constitution WY is backcrossed to a Mexican

male XY, the sex ratio remains one to one, being the same as from the original mating.

When, however, an intervarietal hybrid female of the constitution WX is backcrossed to a Mexican male XY, the sex ratio is three females (WY, WX, XX) to one male (XY).

Histological studies of the differentiating gonads in WY, WX and XX fishes revealed normal ovarian elements. In XY fish, normal testicular features were present. No evidence was found for sex reversals in any intervarietal or backcross hybrids in the present series of experiments.

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EXPLANATION OF THE PLATES

PLATE I

- FIG. 1. The spotted platyfish, *Xiphophorus (Platypoecilus) maculatus* of the domesticated stock 8B. Above, male (Y)Sp(Y)Sp. Below, female (W)Sp/(Y)Sp.
- FIG. 2. The stripe-sided platyfish of the same species, but of the wild stock from the Rio Jamapa, Veracruz, Mexico. Above, male (X)Sr/(Y)Sr. Below, female (X)Sr/(X)Sr.

PLATE II

- FIG. 1. Above, spot-sided female platyfish of the genetic constitution (W)Sp/(X)Sr, Pedigree No. 195a-1. Below, its mate, a stripe-sided male, (X)Sr/(Y)Sr, Pedigree No. 195a-11. They produced the young shown in Figure 2.

- FIG. 2. Members of brood 195²a produced by the pair of platyfish shown in Figure 1. The following frequencies of color patterns and sexes were observed: left, 1 stripe-sided female (X)Sr/(X)Sr; center, 2 spot-sided females (W)Sp/(X)Sr, or (W)Sp/(Y)Sr; right, 1 stripe-sided male (X)Sr/(Y)Sr. Sex ratio: three females to one male.